



Map-based analysis of the tenacious glume gene *Tg-B1* of wild emmer and its role in wheat domestication[☆]



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ABSTRACT

The domestication of wheat was instrumental in spawning the civilization of humankind, and it occurred through genetic mutations that gave rise to types with non-fragile rachises, soft glumes, and free-threshing seed. Wild emmer (*Triticum turgidum* ssp. *dicoccoides*), the tetraploid AB-genome progenitor of domesticated wheat has genes that confer tenacious glumes (*Tg*) that underwent genetic mutations to give rise to free-threshing wheat. Here, we evaluated disomic substitution lines involving chromosomes 2A and 2B of wild emmer accessions substituted for homologous chromosomes in tetraploid and hexaploid backgrounds. The results suggested that both chromosomes 2A and 2B of wild emmer possess genes that inhibit threshability. A population of recombinant inbred lines derived from the tetraploid durum wheat variety Langdon crossed with a Langdon — *T. turgidum* ssp. *dicoccoides* accession PI 481521 chromosome 2B disomic substitution line was used to develop a genetic linkage map of 2B, evaluate the genetics of threshability, and map the gene derived from PI 481521 that inhibited threshability. A 2BS linkage map comprised of 58 markers was developed, and markers delineated the gene to a 2.3 cM interval. Comparative analysis with maps containing the tenacious glume gene *Tg-D1* on chromosome arm 2DS from *Aegilops tauschii*, the D genome progenitor of hexaploid wheat, revealed that the gene inhibiting threshability in wild emmer was homoeologous to *Tg-D1* and therefore designated *Tg-B1*. Comparative analysis with rice and *Brachypodium distachyon* indicated a high level of divergence and poorly conserved colinearity, particularly near the *Tg-B1* locus. These results provide a foundation for further studies involving *Tg-B1*, which, together with *Tg-D1*, had profound influences on wheat domestication.

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1. Introduction

Modern day bread wheat (*Triticum aestivum* L., $2n = 6x = 42$, AABBDD genomes) is a major source of human sustenance and accounts

for about 20% of the caloric intake. Bread wheat arose as the result of two separate amphiploidization events involving three related diploid species. The first event involved hybridization between the wild diploid wheat *Triticum urartu* Tumanian ex Gandylian ($2n = 2x = 14$, AA genome) as the donor of the A genome (Dvorak et al., 1993) and a close relative of *Aegilops speltoides* Tausch ($2n = 2x = 14$, SS genome) as the donor of the B genome (a related but distinct form of the S genome) (Blake et al., 1999; Chalupska et al., 2008; Dvorak and Zhang, 1990; Huang et al., 2002; Salse et al., 2008) giving rise to the tetraploid wheat *Triticum turgidum* ssp. *dicoccoides* (Körn.) Thell ($2n = 4x = 28$, AABB genomes) known as wild emmer. This hybridization event took place in the Fertile Crescent of the Middle East (Luo et al., 2007) about a half million years ago (Chalupska et al., 2008; Huang et al., 2002).

The wild species all had natural seed dispersal mechanisms, namely brittle rachises, which caused the spikelets to disarticulate and fall to the ground, or shatter, upon maturity. About 10,000 years ago, wild emmer underwent a transition from having the shattering trait to a form that had a non-brittle rachis (Nesbitt and Samuel, 1996). This non-shattering type is known as cultivated emmer wheat (*T. turgidum* ssp. *dicoccum* L., $2n = 4x = 28$, AABB genomes). At about the same time, a close diploid relative of *T. urartu* known as *Triticum monococcum* ssp. *aegilopoides* (Link) Thell. ($2n = 2x = 14$, AA genome) underwent a

Abbreviations: LDN, Langdon; LDN521-2A, Langdon — *Triticum turgidum* ssp. *dicoccoides* accession PI 481521 chromosome 2A disomic substitution line; LDN521-2B, Langdon — *Triticum turgidum* ssp. *dicoccoides* accession PI 481521 chromosome 2B disomic substitution line; LDN742-2B, Langdon — *Triticum turgidum* ssp. *dicoccoides* accession PI 478742 chromosome 2B disomic substitution line; LDN15A-2A, Langdon — *Triticum turgidum* ssp. *dicoccoides* accession IsraelA chromosome 2A disomic substitution line; CS, Chinese Spring; CS106-2A, Chinese Spring — *Triticum turgidum* ssp. *dicoccoides* accession TA106 chromosome 2A disomic substitution line; CS106-2B, Chinese Spring — *Triticum turgidum* ssp. *dicoccoides* accession TA106 chromosome 2B disomic substitution line; *Tg*, tenacious glume; *Sog*, soft glume; *Br*, brittle rachis; LSD, least significant difference; QTL, quantitative trait loci; SSR, simple sequence repeat; SNP, single nucleotide polymorphism; EST, expressed sequence tag; STS, sequence-tagged site; RFLP, restriction fragment length polymorphism; RIL, recombinant inbred line; LOD, logarithm of the odds; TC, tentative consensus; ITMI, International Triticeae Mapping Initiative.

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similar transition to acquire a non-brittle rachis, and is known as einkorn wheat (*T. monococcum* ssp. *monococcum* L., $2n = 2x = 14$, $A^{m}A^{m}$). Although cultivated emmer and einkorn had non-brittle rachises thereby allowing early farmers to more easily harvest the grain, the seed for both species was hulled and therefore nonfree-threshing. Nevertheless, the formation of einkorn and cultivated emmer wheat was instrumental in spawning human civilization during the Agricultural Revolution of the Neolithic times, and their cultivation spread throughout the Middle East, Europe, Asia, and northern Africa (Nesbitt and Samuel, 1996).

The second amphiploidization event leading to modern bread wheat occurred about 8000 years ago (Huang et al., 2002). This hybridization event occurred between a subspecies of tetraploid *T. turgidum* (most likely a free-threshing form) and the diploid goatgrass *Aegilops tauschii* Coss. ($2n = 2x = 14$, DD genome) (Kihara, 1944; McFadden and Sears, 1946) thereby giving rise to the hexaploid wheat *T. aestivum* L. with pairs of A, B, and D genomes ($2n = 6x = 42$, AABBDD genomes).

Ultimately, transitions in at least three major genes during the evolution of wheat were needed to spawn fully domesticated wheat. Those three major genes are *Br*, *Tg*, and *q*, which confer a brittle rachis, tenacious glumes, and the nonfree-threshing character, respectively. The genes *Br-A1* and *Br-B1* on chromosomes 3A and 3B confer a brittle rachis in wild emmer wheat (Li and Gill, 2006; Nalam et al., 2006; Watanabe and Ikebata, 2000), and were derived from the A- and B-genome donors, respectively. Mutations at both loci occurred resulting in the non-brittle rachis trait of cultivated emmer wheat. Other studies have also indicated that chromosome 2A of some wild emmer accessions harbors a *Br* gene (Peleg et al., 2011; Peleg et al., 2003), which would suggest that perhaps rachis brittleness is under complex regulation or that the 2A locus represents a second genetic pathway controlling spikelet disarticulation.

A mutation in the *q* locus on chromosome 5AL (*q-A1*) about 10,000 years ago led to the formation of the *Q* allele (*Q-A1*), which confers the free-threshing character and also pleiotropically influences numerous additional traits including spike shape, rachis fragility, glume toughness, plant height and heading time (Faris and Gill, 2002; Faris et al., 2003, 2005; Kato et al., 1999, 2003; Mackey, 1954, 1966; Muramatsu, 1963, 1979, 1985, 1986; Sears, 1956; Simons et al., 2006; Singh, 1969; Watkins, 1940; Zhang et al., 2011). The isolation of *Q* showed that it is a member of the AP2 class of transcription factors (Faris et al., 2003; Simons et al., 2006) and related to genes such as *APETALA2* in *Arabidopsis* (Jofuku et al., 1994) and *indeterminate spikelet1* in maize (Chuck et al., 1998). The mutation from *q-A1* to *Q-A1* occurred only once, most likely in the tetraploid *T. turgidum* ssp. involved in the amphiploidization event with *A. tauschii* that formed hexaploid wheat (Simons et al., 2006). In the presence of the mutated forms of *br* and *tg*, *Q-A1* confers a fully tough rachis and free-threshing seed, which is necessary for today's durum (*T. turgidum* ssp. *durum*) and bread wheat (*T. aestivum* ssp. *aestivum*) varieties. The *q-B1* and *q-D1* alleles on homoeologous chromosomes 5B and 5D also contribute to the domestication syndrome, but to a minor extent compared to *Q-A1* because they did not undergo hyperfunctionalization, as did *Q-A1* (Zhang et al., 2011).

In addition to *Q-A1*, genes governing tenacious glumes (*Tg*) also control threshability. The tenacious glume trait was first described by Kerber and Dyck (1969) who attributed the character to an incompletely dominant gene. The gene, now designated as *Tg-D1*, was first mapped using cytogenetic stocks to the short arm of chromosome 2D by Kerber and Rowland (1974). More recent experiments have validated the position of *Tg-D1* using segregating mapping populations and molecular markers (Dvorak et al., 2012; Jantasuriyarat et al., 2004; Nalam et al., 2007; Sood et al., 2009). *Tg-D1* is epistatic to *Q-A1* because plants that have both are nonfree-threshing (Kerber and Rowland, 1974), and both *Tg-D1* and *Q-A1* are required for the free-threshing trait. Therefore, the first hexaploid forms were not free-threshing due to acquisition of *Tg-D1* from *A. tauschii* even though they harbored *Q-A1*. The *Tg-D1*

gene presumably underwent mutation to *tg-D1* very rapidly giving rise to fully domesticated free-threshing bread wheat.

The spikes of einkorn and emmer wheat are also nonfree-threshing due to tough adherent glumes that hold the kernels tightly. Evaluation of a free-threshing mutant of einkorn wheat, known as *Triticum sinskajae*, revealed that a single gene designated *Sog* on the short arm of chromosome 2A^m conferred the soft glume trait (Sood et al., 2009; Taenzler et al., 2002). Comparative mapping analysis with *Tg-D1* indicated that the two genes are not homoeologous and therefore probably represent different genetic pathways (Sood et al., 2009).

Simonetti et al. (1999) used a *T. turgidum* ssp. *dicoccoides* (wild emmer) × *T. turgidum* ssp. *durum* (durum wheat) mapping population to identify quantitative trait loci (QTLs) associated with threshability. The *Q-A1* locus accounted for one QTL, and a second QTL with major effects was identified on the short arm of 2B. This result suggested that wild emmer wheat harbored a gene conferring glume tenacity on 2BS perhaps homoeologous to *Tg-D1*, which has been inferred in recent work (Dvorak et al., 2012). However, the relationship of *Tg-B1* on 2BS with *Tg-D1* on 2DS and *Sog* on 2A^mS has not yet been determined conclusively.

The objectives of the research were to evaluate the effects of wild emmer chromosome 2B on threshability in the free-threshing backgrounds of the durum wheat variety Langdon (LDN) and the bread wheat cultivar Chinese Spring (CS), to map the chromosomal location of the *Tg-B1* gene on 2B, and to determine homoeologous relationships of *Tg-B1* with *Sog* and *Tg-D1*. The results of this research provide further clues into the events shaping the domestication of both tetraploid and hexaploid wheat.

2. Materials and methods

2.1. Plant materials

The durum wheat variety LDN, two LDN-*T. turgidum* ssp. *dicoccoides* chromosome 2B disomic substitution lines, and two LDN-*T. turgidum* ssp. *dicoccoides* chromosome 2A disomic substitution lines were evaluated for threshability. These tetraploid substitution lines involved substituting the *T. turgidum* ssp. *dicoccoides* chromosomes for a native pair of homologous LDN chromosomes. The chromosome 2A substitutions were derived from *T. turgidum* ssp. *dicoccoides* accessions PI 481521 and IsraelA, and are designated LDN521-2A and LDNIsA, respectively. The chromosome 2B substitutions were derived from accessions PI 478742 and PI 481521 and designated LDN742-2B and LDN521-2B, respectively. These lines were developed by Dr. L.R. Joppa (USDA-ARS, retired) as described in Xu et al. (2004). In addition, the hexaploid wheat cultivar CS and CS disomic substitution lines involving *T. turgidum* ssp. *dicoccoides* chromosomes 2A and 2B derived from accession TA106 (CS106-2A and CS106-2B) were also evaluated for threshability. These lines were developed by the late E.R. Sears and obtained from the Wheat Genetics Resource Center, Kansas State University, Manhattan, KS.

The population used to map the tenacious glume trait consists of 152 recombinant inbred lines (RILs) derived from the cross between LDN and LDN521-2B. Details on the development of the population will be described elsewhere (S. Chao and M.E. Sorrells, personal communication). Representative spikes of the parents and PI 481521, the chromosome 2B donor, are shown in Fig. 1. This population is homozygous for all chromosomes with the exception of 2B making it ideal for the analysis of traits governed by genes on 2B in an otherwise homogeneous background.

2.2. Phenotypic and statistical analysis

The wheat lines CS, CS106-2A, CS106-2B, LDN, LDNIsA-2A, LDN521-2A, LDN521-2B, and LDN742-2B were planted in six-inch clay pots in the greenhouse with an average temperature of 21 °C and a 16-h



Langdon LDN-DIC 2B(521) PI 481521

Fig. 1. Mature spikes of Langdon (left), the Langdon – *Triticum turgidum* ssp. *dicoccoides* accession PI 481521 chromosome 2B disomic substitution line (middle), and *Triticum turgidum* ssp. *dicoccoides* accession PI 481521 (right).

photoperiod. Each pot contained one plant and constituted an experimental unit. The experiment consisted of a total of five replicates arranged in a completely randomized design. Wheat spikes were harvested at maturity and placed in a dryer at 37 °C overnight before threshing and two to four spikes per plant were evaluated. Ease of threshing was analyzed as described in Sood et al. (2009) with a few modifications. Each spike was placed on a corrugated rubber mat lying on a bench, and a wooden block covered with the same corrugated rubber material was rubbed back and forth across the spike one

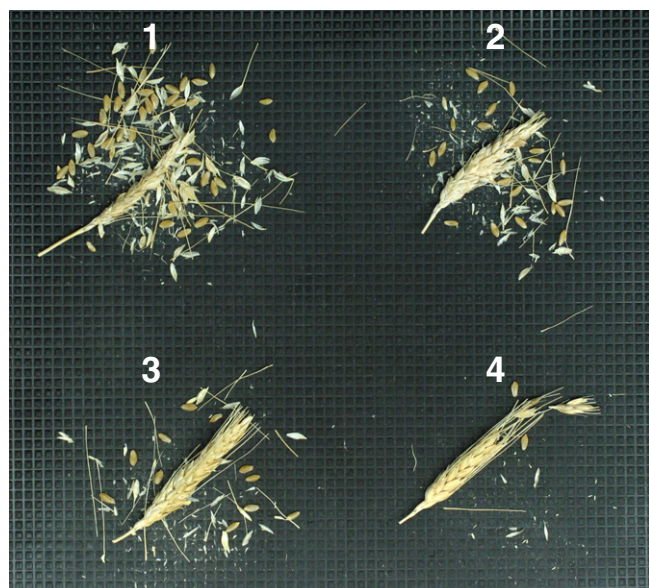


Fig. 2. The scale used to classify seed threshability of the LDN × LDN521-2B recombinant inbred lines where 1 = completely free-threshing with virtually all seed released from the hulls; 2 = mostly free-threshing with a minor portion (<50%) of the seed remaining hulled; 3 = somewhat difficult to thresh with a major portion (>50%) of the seed remaining hulled; and 4 = difficult to thresh with only a few seeds being released from the hulls.

Table 1

Average threshability scores in Chinese Spring, Langdon, and disomic chromosome substitution lines involving chromosomes 2A and 2B from *Triticum turgidum* ssp. *dicoccoides*.

Line	Threshability score ^a
Langdon	1.33ab
LDNsA-2A	2.95c
LDN742-2B	3.50cd
LDN521-2A	3.83d
LDN521-2B	3.45cd
Chinese Spring	1.00a
CS106-2A	2.00b
CS106-2B	1.00a

^a Average threshability scores based on a 1–4 scale where 1 = most easily threshed and 4 = most difficult to thresh. Numbers followed by the same letter within the same column are not significantly different at the 0.05 level of probability as determined by least significant difference (LSD).

time only. The threshability of each spike was rated on a 1–4 scale, where 1 = completely free-threshing with virtually all seed released from the hulls; 2 = mostly free-threshing with a minor portion (<50%) of the seed remaining hulled; 3 = somewhat difficult to thresh with a major portion (>50%) of the seed remaining hulled; and 4 = difficult to thresh with only a few seeds being released from the hulls (Fig. 2). The same person threshed all spikes in the experiment so to reduce variability in persons performing the exercise.

A total of three replicates arranged in a completely randomized design were evaluated for the LDN × LDN521-2B RIL population. Plants were grown in clay pots with one plant per pot under the same conditions as described above. Four main spikes from each plant were evaluated for threshability as described above. The scores for spikes from the same plant were averaged to derive the mean score for each plant within a replicate. Phenotypic scores for each replicate were combined to obtain the overall mean for each RIL. Fisher's protected least significant difference (LSD) was calculated to identify significant differences among means at the 0.05 level of probability. Plants were also scored for glaucousness, which segregated as a Mendelian trait and used as a marker in the assembly of the chromosome 2B linkage map.

Bartlett's Chi-squared test was conducted using PROC GLM in SAS program version 9.3 (SAS Institute 2011) to determine homogeneity of error variances among replicates. Because error variances among replicates were homogeneous (data not shown), the values for threshability for each replicate were combined to derive the overall means. Fisher's least significant difference (LSD) test was used to determine significant differences among means of the RILs at the 0.05 level of

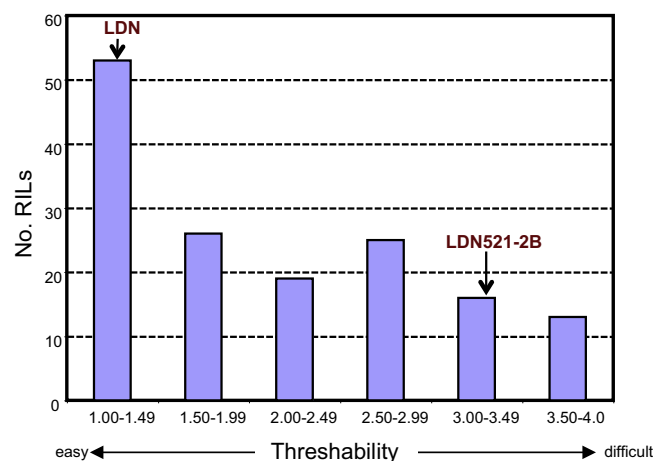


Fig. 3. Histogram for ease of threshability in the LDN × LDN521-2B recombinant inbred population.

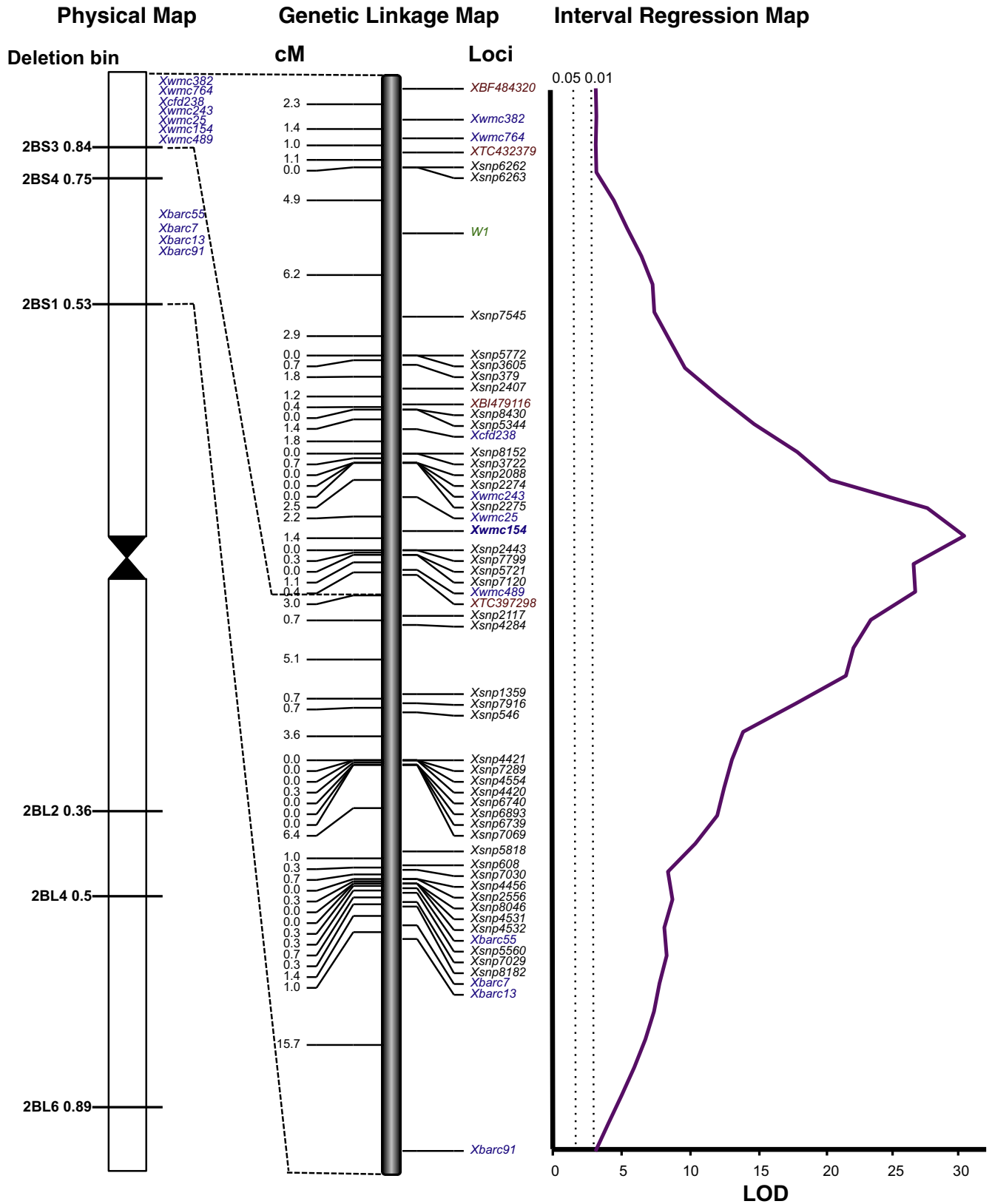


Fig. 4. Mapping of the threshability trait on wheat chromosome 2B. Left: the deletion-based physical map. Deletion line designations and their chromosome fraction breakpoints are indicated along the left side of the physical map, and bin-mapped SSR markers are shown along the right. Middle: the genetic linkage map constructed in the LDN × LDN521-2B recombinant inbred population with SSRs (blue), SNPs (black), and EST-STS (red) markers shown along the right and centiMorgan distances between markers shown along the left. Dotted lines connecting the genetic linkage map to the physical map indicate approximate positions of deletion breakpoints on the linkage map. Right: interval regression map of 2BS in the LDN × LDN521-2B recombinant inbred population. A logarithm of the odds (LOD) scale is shown along the x-axis. Significance thresholds at the 0.05 and 0.01 levels of probability are indicated by the vertical dotted lines. The marker *Xwmc154* is the marker closest to the peak of the interval regression line and is shown on the linkage map in bold.

probability. The means of the RILs were used to conduct QTL analysis using simple interval regression mapping with the software program QGene v4.3.10 (Joeahanes and Nelson, 2008). A permutation test of 1000 iterations revealed critical LOD thresholds of 1.8 and 2.6 for the 0.05 and 0.01 levels of probability, respectively, in the LDN × LDN521-2B population.

2.3. Genotyping and linkage analysis

DNA was isolated from juvenile plants of LDN, LDN521-2B, and the 152 RILs of the population as described previously (Faris et al., 2000) for analysis of simple sequence repeat (SSR) and expressed-sequence tagged (EST)-based PCR markers. A total of 46 SSR primer pairs derived from BARC (Cregan and Song, <http://www.scabusa.org>), GWM (Röder et al., 1998a,b), GDM (Pestsova et al., 2000), CFA (Sourdille et al., 2003), and WMC (Somers et al., 2004) sets and known to detect loci on wheat chromosome 2B were then used to amplify the fragments from LDN and LDN521-2B using PCR conditions described by Röder et al. (1998b).

Sequences for ESTs that mapped to the most distal deletion bin of the short arm of chromosome 2B (Qi et al., 2004) were downloaded (<http://www.wheat.pw.usda.gov/index.shtml>) and used to design primers for amplification by PCR using the program Primer 3 (Rozen and Skaletsky, 2000). EST-derived primer sets showing polymorphism between LDN and LDN521-2B were then used to amplify the fragments from the RIL population for mapping. Electrophoresis and fragment visualization were done as described in Abeysekara et al. (2010).

The single nucleotide polymorphism (SNP) markers used for mapping were from a design containing the SNPs derived from the wheat 9K SNP array (Cavanagh et al., 2013). Details on developing the SNP design and SNP genotyping will be described elsewhere (S. Chao and M.E. Sorrells, personal communication).

The linkage map of chromosome 2B was assembled using the program MapDisto 1.7 (Lorieu, 2012). The 'find groups' command with an LOD > 3.0 and a maximum theta of 0.30 was used to verify linkage of markers. The 'order sequence' command was used to establish the initial marker order followed by 'check inversions', 'ripple order', and 'drop locus' to determine the best map. Map distances were determined using the Kosambi mapping function (Kosambi, 1944).

2.4. Comparisons with rice and *Brachypodium distachyon* sequences

Comparisons of mapped marker sequences with rice and *B. distachyon* sequences were done similar to that described in Lu and Faris (2006). Briefly, sequences of ESTs representing the mapped markers were downloaded from the NCBI database (<http://www.ncbi.nlm.nih.gov/>) and tested for similarity to sequences in the wheat gene index database release 12.0 at the Dana Farber Cancer Institute (<http://compbio.dfci.harvard.edu/cgi-bin/tgi/gimain.pl?gudb=wheat>) to identify tentative consensus (TC) sequences. Tentative consensus or EST sequences representing all mapped EST-based and SNP markers were subjected to BLASTn and tBLASTx (Altschul et al., 1997) searches of the rice genome using the Rice Genome Annotation Project Website (<http://rice.plantbiology.msu.edu/>; MSU Rice Genome Annotation Release 7) and of the *B. distachyon* genome using Gramene (Ware et al., 2002; <http://www.gramene.org>; Release 38, *B. distachyon* genome assembly v1.0). Thresholds for declaring significant matches were as described in Lu and Faris (2006). Results regarding significant matches to rice and *B. distachyon* orthologs were verified using the Wheat Zapper (Alnemer et al., 2013).

3. Results

3.1. Threshability of genetic stocks

LDN produced spikes with seeds that were easily threshed resulting in a mean threshability score of 1.33 (Table 1). Compared to LDN, all the LDN-based genetic stocks had spikes with seeds that were significantly more difficult to thresh. The LDN521-2A line was the most difficult to thresh with a mean threshability score of 3.83, and it was more difficult to thresh than the other *T. turgidum* ssp. *dicoccoides* chromosome 2A substitution line, LDN15A, which had a mean threshability score of 2.95. The two stocks with chromosome 2B substitutions, LDN742-2B and LDN521-2B, had nearly identical threshability scores of 3.50 and 3.45, respectively. These did not differ significantly from either of the chromosome 2A substitution lines. This data suggests that chromosomes 2A from *T. turgidum* ssp. *dicoccoides* accessions IsraelA and PI 481521 and 2B from accessions PI 481521 and PI 478742 all possess genes that inhibit threshability in the LDN durum background.

Chinese Spring and CS106-2B were the most free-threshing of all with both having a mean threshability score of 1.00 (Table 1). The line CS106-2A was slightly more difficult to thresh with a mean of 2.00 indicating that chromosome 2A from the *T. turgidum* ssp. *dicoccoides* accession TA106 possesses factor(s) that inhibit threshability in the CS background.

3.2. Phenotypic analysis of the LDN × LDN521-2B recombinant inbred population

The population of RILs derived from LDN × LDN521-2B segregated for threshability with mean scores ranging from 1.00 to 3.92 (Fig. 3). The overall mean threshability score of the population was 2.10. No RIL was easier to thresh than LDN, and no RIL was significantly ($P < 0.05$) more difficult to thresh than LDN521-2B. Therefore, transgressive segregants were not observed. For plant glaucousness, the population segregated in a ratio of 80:72 for glaucous:nonglauous. This ratio was not significantly different than what is expected for a single gene (Chi-squared = 0.42 df = 1; $0.75 < P < 0.50$).

3.3. Genetic linkage mapping, physical mapping, and QTL analysis

The genetic linkage map assembled in the LDN × LDN521-2B population consisted of 58 markers that spanned a genetic distance of 79.5 cM (Fig. 4), and represents the short arm of chromosome 2B. Fifty-seven of the 58 markers were DNA markers. Of these, four were EST-based, 11 were SSRs, and 42 were SNP markers. Plant glaucousness was mapped as a morphological marker (W1) and constituted the 58th marker on the map (Fig. 4).

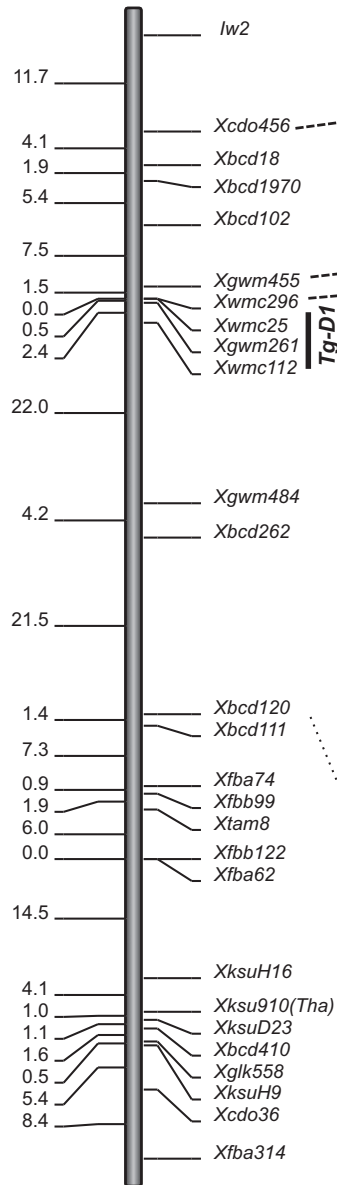
Interval regression analysis of the mean threshability data revealed a major QTL on chromosome arm 2BS in the LDN × LDN521-2B population (Fig. 4). The QTL peaked at the marker *Xwmc154*, had a LOD of 30.0, and explained 59.7% of the variation ($R^2 = 0.597$).

The SSR markers that were used in the construction of the LDN × LDN521-2B 2BS map were placed on the 2B deletion based physical map. The SSR markers *Xwmc489*, *Xwmc154*, and all other SSR markers that mapped distal to these on the linkage map were located in the most distal deletion bin on 2BS (Fig. 4). This indicated that the gene governing the threshability QTL was located in the distal 26% of the short arm of chromosome 2B.

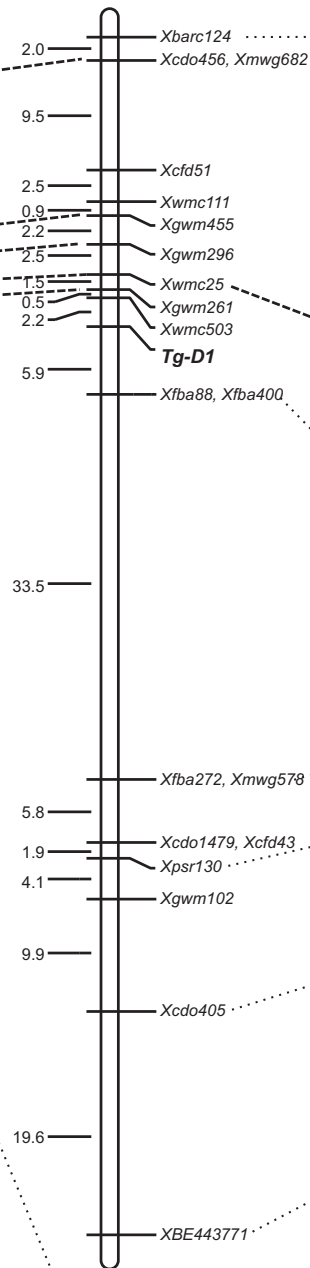
To determine if threshability could be analyzed and mapped as a Mendelian trait, we classified the phenotypic scores based on the 1–4 threshability scale into discreet free-threshing and nonfree-

Fig. 5. Comparative mapping of *Tg-B1*, *Tg-D1*, and *Sog*. Maps of wheat chromosome 2D developed in the ITMI population (from Nalam et al., 2007) and the TA2987 × TA3419 population (from Sood et al., 2009) show the location of the *Tg-D1* locus. The chromosome 2B map developed in the LDN × LDN521-2B population developed in this research shows the location of *Tg-B1*, and the chromosome 2A³ map (from Sood et al., 2009) shows the location of the *Sog* locus. Dotted lines connect common loci among the maps.

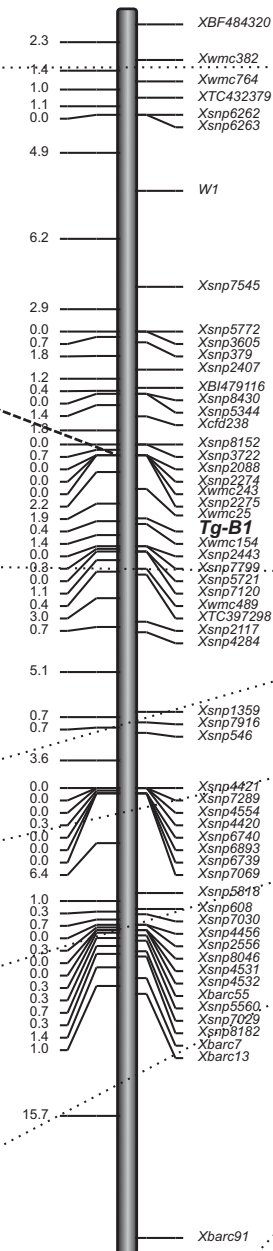
Chromosome 2D (ITMI)



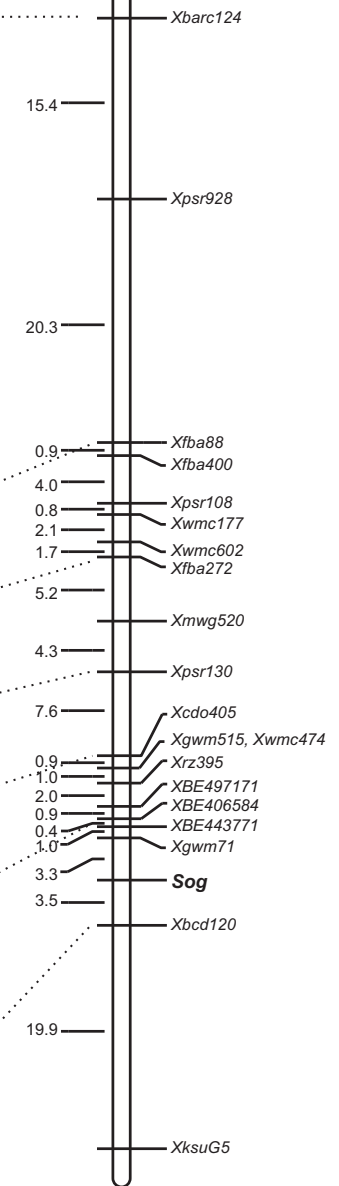
Chromosome 2D (TA2987 x TA3419)



Chromosome 2B (LDN x LDN521-2B)



Chromosome 2A^m



threshing classes where RILs with mean scores less than 2.0 were considered free-threshing and RILs with means of 2.0 or greater were considered nonfree-threshing. Subsequent genetic linkage analysis resulted in the threshability locus mapping between markers *Xwmc154* and *Xwmc25* flanked by genetic distances of 0.4 and 1.9 cM, respectively (Fig. 5). This gene at this locus will hereafter be referred to as *Tg-B1*.

3.4. Comparative mapping of *Tg-B1* with *Tg-D1* and *Sog*

The *Tg-D1* gene (formerly *Tg1*) on chromosome arm 2DS in *A. tauschii* confers a very tenacious glume and severely inhibits threshability in *A. tauschii* as well as in synthetic hexaploid wheat lines constructed from *T. turgidum* (AABB genomes) \times *A. tauschii* (DD genome) crosses. The *Tg-D1* gene has been mapped in several populations derived from synthetic hexaploid wheat \times *T. aestivum* crosses included the ITMI population (Nalam et al., 2007) and a population derived from Canthatch (TA2987) \times a synthetic hexaploid developed by crossing the extracted tetraploid of Canthatch to *A. tauschii* (TA3419) (Sood et al., 2009). In the ITMI population, *Tg-D1* was mapped as a QTL near the SSR marker *Xwmc25* on 2DS (Fig. 5). Similarly, in the Canthatch-derived population, the *Tg-D1* locus mapped just 4.2 cM proximal to *Xwmc25* on 2DS. In comparison, we found that the *Tg-B1* locus mapped 1.9 cM proximal to *Xwmc25* on the short arm of chromosome 2B in the LDN \times LDN521-2B population. Assuming that the SSR marker *Xwmc25* detects homoeoloci on chromosomes 2B and 2D, these results strongly indicate that *Tg-B1* and *Tg-D1* are indeed homoeologous genes. On the contrary, Sood et al. (2009) showed that the *Sog* gene from *T. monococcum* chromosome arm 2A^mS was not homoeologous to *Tg-D1* because *Sog* mapped in a more proximal position compared to *Tg-D1*. Therefore, this would indicate that *Tg-B1* is not homoeologous to *Sog* either (Fig. 5).

3.5. Synteny of the *Tg-B1* region with rice and *B. distachyon*

Comparisons with rice genomic sequences indicated that, of the 46 SNP and EST-based markers mapped on 2BS in the LDN \times LDN521-2B population, 15 and 9 had significant similarity to genes on rice chromosomes 7 and 4, respectively (Fig. 6, Supplementary Table 1). Thirteen marker sequences had similarity to sequences on rice chromosomes 1, 2, 3, 5, 11, and 12, and 9 marker sequences had no significant matches to sequences in the rice genome. Colinearity with rice chromosome 7 was well conserved in the proximal portion of wheat 2BS where ten markers were perfectly colinear. The distal half of 2BS, including the *Tg-B1* locus, was much less conserved with many breaks in colinearity evident including numerous inversions and rearrangements relative to both rice chromosomes 7 and 4. No apparent syntenic region for the *Tg-B1* locus could be detected in the rice genome.

Comparisons of the wheat 2BS marker sequences with *B. distachyon* indicated slightly better levels of synteny compared to rice. Twenty-two and 13 marker sequences had significant similarity to sequences on *B. distachyon* chromosomes 1 and 5, respectively (Fig. 6, Supplementary Table 1). Four sequences had similarity to *B. distachyon* chromosome 2 sequences and one had similarity to a *B. distachyon* sequence on chromosome 3. Only five of the marker sequences had no similarity to any *B. distachyon* sequences. Similar to the situation with rice, the proximal portion of the LDN \times LDN521-2B map was highly conserved with *B. distachyon* where fifteen markers were colinear with genes on

B. distachyon chromosome 1. However, the distal half of 2BS was highly rearranged relative to *B. distachyon* chromosomes 1 and 5, and no apparent colinearity encompassing the *Tg-B1* locus was detected.

4. Discussion

Since the report of a QTL on chromosome arm 2BS associated with seed threshability in a durum wheat \times wild emmer wheat population by Simonetti et al. (1999), speculations have been made regarding whether or not the underlying gene was homoeologous to *Tg-D1*, but studies to investigate the relationship directly have not been conducted. Therefore, the primary objectives of this research were to map the gene inhibiting threshability on chromosome 2B in *T. turgidum* ssp. *dicoccoides* accession PI 481521, and to determine if it was homoeologous to the long-recognized tenacious glume gene *Tg-D1* on chromosome 2D. The map generated in the LDN \times LDN521-2B population of chromosome arm 2BS consisted of SNPs, EST-based PCR markers, and SSRs, and had very few markers in common with previously developed maps of chromosome 2D used for mapping the *Tg-D1* locus because the latter were constructed with primarily restriction fragment length polymorphism (RFLP) markers. However, the SSR marker *Xwmc25*, which mapped 1.9 cM distal to *Tg-B1* in the LDN \times LDN521-2B population, was also shown to detect a locus tightly linked on the distal side of *Tg-D1* on chromosome 2D in the ITMI (Nalam et al., 2007), TA2987 \times TA3419 (Sood et al., 2009), and CS \times DSA5403 (CS2D) (Dvorak et al., 2012) populations. It is highly likely that *Xwmc25* detects homoeoalleles on chromosomes 2B and 2D, which is a strong indication that *Tg-B1* and *Tg-D1* are homoeologous genes, and they are therefore named as such.

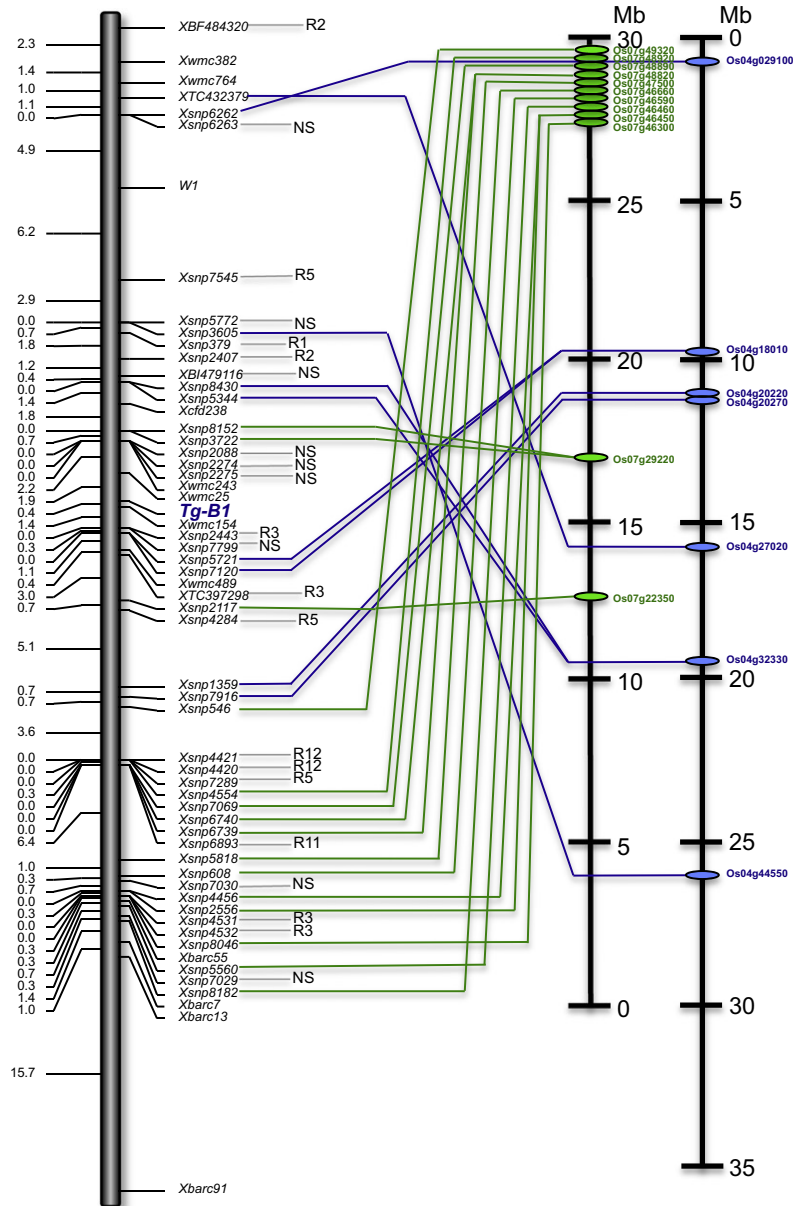
Sood et al. (2009) demonstrated that *Tg-D1* was not homoeologous to *Sog* on *T. monococcum* 2A^m because *Sog* was located more proximal relative to *Tg-D1*. Indirect comparisons between the LDN \times LDN521-2B map and the *T. monococcum* 2A^m map generated by Sood et al. (2009) using the TA2987 \times TA3419 2D map as an intermediate also indicates *Sog* maps proximal relative to *Tg-B1*. Therefore, whereas *Tg-B1* and *Tg-D1* are homoeologous, *Sog* is not and represents a unique locus relative to the *Tg* set.

The map of chromosome 2B generated by Simonetti et al. (1999) that was used to identify a QTL associated with threshability consisted of only RFLP markers. Therefore, it is impossible to make direct comparisons between their map and our map. However, because their map was constructed in a durum \times wild emmer population, it is highly likely that the 2BS locus reported in their research is the same as the *Tg-B1* locus reported here.

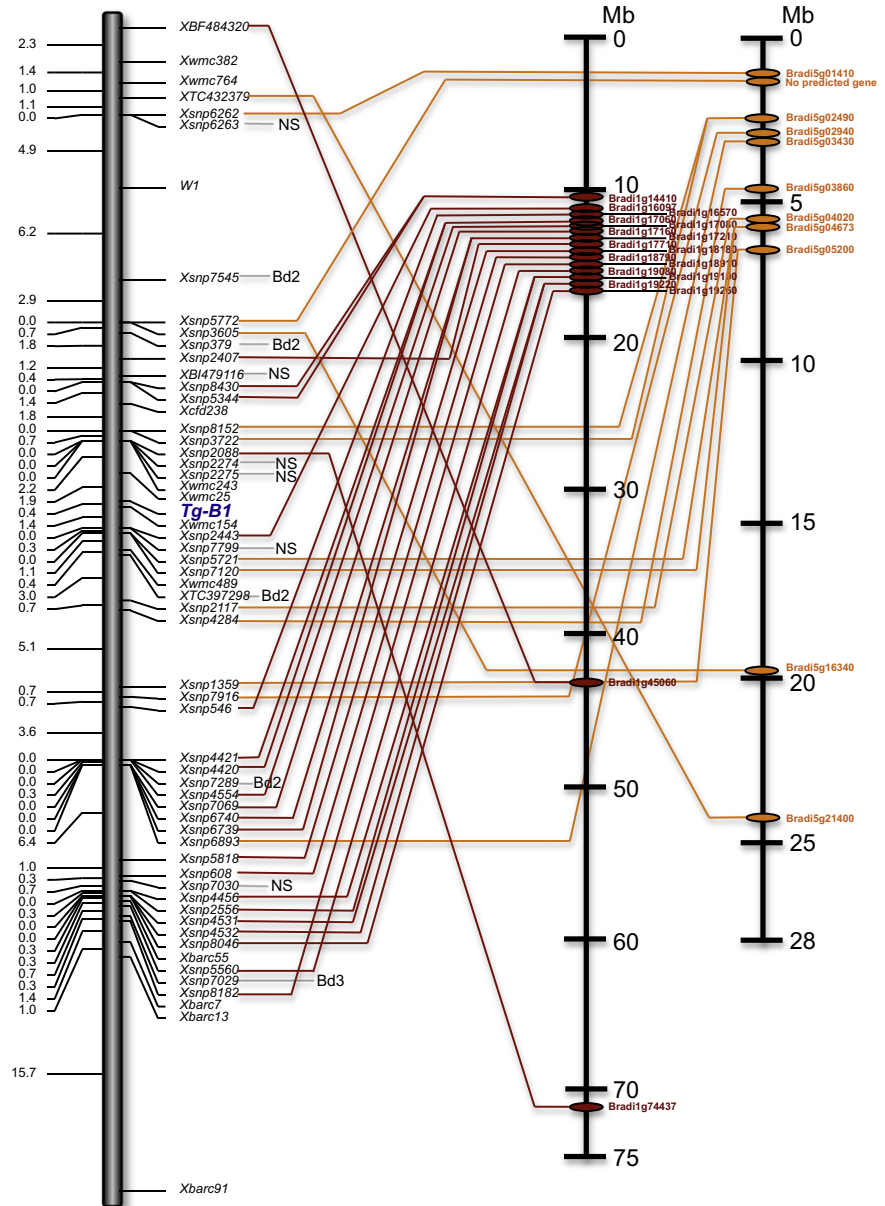
It is unknown if *Tg-B1* is universal among wild emmer wheat accessions, or if allelic variation may exist. Evaluations of the LDN – *T. turgidum* ssp. *dicoccoides* chromosome 2B substitution lines suggested that accession PI 478742 harbors the *Tg-B1* allele for tenacious glumes because LDN742-2B was as difficult to thresh as LDN521-2B. These results also indicate that, like *Tg-D1*, *Tg-B1* is epistatic to *Q-A1* because both LDN742-2B and LDN521-2B harbor the *Q-A1* allele. On the contrary, the CS – *T. turgidum* ssp. *dicoccoides* substitution line involving TA106 chromosome 2B was as free threshing as CS. CS is a hexaploid wheat whereas LDN is a tetraploid, and it is possible that the difference in ploidy level could affect the expression of the gene such that *Tg-B1* is not epistatic to *Q-A1* in the CS background and therefore does not confer the tenacious glume phenotype. However, this would argue against the findings of Dvorak et al. (2012) who reported that factor(s) on 2BS

Fig. 6. Comparative mapping of the map of chromosome arm 2BS developed in the LDN \times LDN521-2B population with the rice (left) and *Brachypodium distachyon* (right) genomes. Left: Wheat EST and SNP marker sequences with significant similarity to sequences on rice chromosomes 7 and 4 are indicated by the green and blue lines, respectively, showing the positions of putative orthologous genes on the respective rice chromosomes. The rice gene names and megabase scales are indicated to the right of the rice maps. Wheat marker sequences with homology to rice chromosomes other than 4 and 7, or that had no significant similarity to any rice sequences, are indicated with gray lines followed by the rice chromosome designation or 'NS' (no significance). Right: Similarly, wheat EST and SNP marker sequences with significant similarity to sequences on *B. distachyon* chromosomes 1 and 4 are indicated by the dark red and orange lines, respectively, showing the positions of putative orthologous genes on the respective *B. distachyon* chromosomes. The *B. distachyon* gene names and megabase scales are indicated to the right of the *B. distachyon* maps. Wheat marker sequences with homology to *B. distachyon* chromosomes other than 1 and 5, or that had no significant similarity to any *B. distachyon* sequences, are indicated with gray lines followed by the *B. distachyon* chromosome designation or 'NS' (no significance).

LDN × LDN521-2B



LDN × LDN521-2B



inhibited threshability in hexaploid backgrounds. A more plausible explanation is that TA106 lost the *Tg-B1* gene due to mutation or through gene flow, which has occurred among wild emmer and domesticated tetraploid and hexaploids (Berkman et al., 2013; Dvorak et al., 2006; Luo et al., 2007).

To our knowledge, there have been no published reports regarding gene(s) on wheat chromosome 2A that confer a tenacious glume and/or a nonfree-threshing phenotype (although some indirect evidence is provided by Dvorak et al., 2012). Here, we found chromosome 2A from both *T. turgidum* ssp. *dicoccoides* accessions IsraelA and PI 481521 conferring a nonfree-threshing phenotype on the LDN genetic background. In addition, the CS – *T. turgidum* ssp. *dicoccoides* accession TA106 chromosome 2A substitution line was significantly more difficult to thresh than CS. These findings suggest that wild emmer accessions likely harbor genes on chromosome 2A in addition to the *Tg-B1* gene on 2B that inhibit threshability, and it is possible that it is due to a gene homoeologous to *Tg-B1* and *Tg-D1*, i.e. *Tg-A1*. Further experimentation and genetic studies are needed to test this hypothesis.

Comparisons of the 2BS genetic linkage map constructed in the LDN × LDN521-2B population with rice and *B. distachyon* genomic sequences revealed good levels of colinearity between the proximal region of 2BS with rice chromosome 7 and *B. distachyon* chromosome 1. The more distal region of 2BS consisted of marker sequences with colinearity to rice chromosomes 7 and 4, and to *B. distachyon* chromosomes 1 and 5, and colinearity of the sequences was much less conserved compared to the proximal region due to numerous apparent rearrangements. These findings largely agree with previous studies involving the comparative analysis of wheat chromosomes with genomic sequences of rice and *B. distachyon* (Conley et al., 2004; Luo et al., 2009, 2013; Sorrells et al., 2003), and tend to reflect the evolutionary divergence that has occurred between wheat and the model monocot species rice and *B. distachyon* with the latter being more closely related to wheat than the former. In addition, these findings show that neither model species is likely to be useful for further genomic analysis or map-based cloning of *Tg-B1* because the locus lies within a region that is highly rearranged in both rice and *B. distachyon*.

The transition of wild emmer wheat to cultivated emmer wheat primarily involved the acquisition of a non-brittle rachis by the latter. However, both forms of emmer have non-free threshing seed, but all the factors governing this trait in these two forms have not been completely characterized. We know that wild emmer has the *Tg-B1* allele for tenacious glumes and also the *q-A1* allele. It is also possible that wild emmer possesses a *Tg-A1* allele, as suggested by the results of this work and that of Dvorak et al. (2012), which would increase glume tenacity and further inhibit threshability. Therefore, as it pertains to seed threshability, wild emmer likely has the genotype *Tg-A1TgA1/Tg-B1TgB1/q-A1q-A1*.

Although the seed of cultivated emmer is also non-free threshing, it is not as difficult to thresh as that of wild emmer. Cultivated emmer is known to harbor the *q-A1* allele, which in itself could confer non-free threshing seed, but it is not known whether or not cultivated emmer might possess *Tg-A1*, *Tg-B1*, or both alleles, or if the differences in glume tenacity and threshability between wild and cultivated emmer might be due to other genes. Fully domesticated tetraploid wheat (e.g. *T. turgidum* ssp. *durum*), on the other hand, would necessarily have the genotype *tg-A1tg-A1/tg-B1tg-B1/Q-A1Q-A1* because it has a fully tough rachis and the seed is completely free threshing. Therefore, the transition from wild emmer to a fully domesticated form required mutations to occur in *Tg-A1*, *Tg-B1*, and *q-A1*, and while it is known that the mutation of *q-A1* to *Q-A1* likely occurred during the transition of cultivated emmer to free-threshing tetraploid wheat, it is unknown yet if the mutations from *Tg-A1* to *tg-A1* and *Tg-B1* to *tg-B1* occurred during the transition of wild to cultivated emmer, or from cultivated emmer to the fully domesticated tetraploid form. Additional genetic and molecular studies are needed to determine if cultivated emmer possesses *Tg-A1*, *Tg-B1*, or both.

It is most likely that the tetraploid *T. turgidum* ssp. involved in the amphiploidization event with *A. tauschii* was a fully free-threshing form for reasons argued by Dvorak et al. (2012) and Faris (2014). The *A. tauschii* accession involved in the hybridization event would have contributed a *Tg-D1* allele thus making the original *T. aestivum* hexaploid non-free threshing (Kerber and Rowland, 1974). The *Tg-D1* allele likely underwent mutation to *tg-D1* very rapidly because the primitive form does not exist in nature today and non-free threshing forms of *T. aestivum* are absent from the archeological record prior to the occurrence of free-threshing hexaploids. It was previously thought that non-free threshing hexaploid forms known as European and Asian spelta (*T. aestivum* ssp. *spelta* L., $2n = 6x = 42$, AABBDD genomes) might be progenitors of free-threshing bread wheat. However, it is now known that European spelta arose as a result of hybridization between domesticated hexaploid wheat (subspecies *compactum*) and cultivated emmer wheat (Blatter et al., 2002, 2004; MacKey, 1966; Yan et al., 2003). Asian spelta also likely arose more recently through hybridization of domesticated hexaploid wheat (subspecies *aestivum*) and either wild or cultivated emmer (Dvorak et al., 2012). Dvorak et al. (2012) indicated that most Asian spelta forms are non-free threshing (despite having *Q-A1*) due to possession of the *Tg-B1* allele (as opposed to *Tg-D1*), which they likely acquired as a result of the hybridization with hulled emmer wheat. Therefore, both *Tg-B1* and *Tg-D1* alleles have had significant impacts on wheat evolution and domestication, and they are of particular interest for gaining insights into the events that shaped our modern day tetraploid and hexaploid wheats.

Conflict of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

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